Does a giant tortoise taxon substitute enhance seed germination of exotic fleshy-fruited plants?

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Running title: Taxon substitute and germination of exotic plants

Does a giant tortoise taxon substitute enhance seed germination of exotic fleshy-fruited plants?

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Abstract

Aims The use of exotic species as taxon substitutes to restore lost ecological interactions is currently hotly debated. *Aldabrachelys gigantea* giant tortoises have recently been introduced to three islands in the Mascarene archipelago (Ile aux Aigrettes, Round Island and Rodrigues) to resurrect herbivory and seed dispersal functions once performed by extinct giant tortoises. However, potential unintended impacts by frugivore substitutes on native ecosystems, for example whether they will facilitate the germination of exotic plant species, are largely unknown. We investigated whether *A. gigantea* introduced to Rodrigues in 2006 could enhance the germination percentage of four widespread fleshy-fruited exotic species on the island. Using germination trials to forecast unintended impacts that could arise from the introduction of a frugivorous taxon substitute enables conservation managers to limit potential adverse negative interactions before they occur.

Methods In germination trials that ran over four months, we investigated the effects of ingestion (gut passage and deposition in faeces) by sub-adult and adult *A. gigantea* on the germination percentage of four exotic fleshy-fruited plant species introduced to Rodrigues. We fed fruits of these plant species to sub-adult and adult *A. gigantea* to test how variation in age and size of the frugivore would affect seed germination. Feeding of distinctly coloured plastic pellets together with the fruits allowed us to test for individual tortoise effects on seed germination.

Important Findings Ingestion by *A. gigantea* increased the percentage of seeds germinating of *Mimusops coriacea* and *Lantana camara,* but not percentage germination of *Veitchia merrillii* or *Wikstroemia indica*. Seeds were more likely to germinate following ingestion by sub-adult rather than adult tortoises, which may be a consequence of the shorter gut-passage time observed for sub-adults. Our results

demonstrate that introduced frugivorous taxon substitutes could facilitate germination of exotic and invasive plants and highlight the need for conservation managers to weigh the risk of taxon substitutes potentially facilitating the germination and recruitment of exotic fleshy-fruited plants against the benefit of restoring lost seed dispersal functions of threatened indigenous plants. Our findings also highlight the importance of considering age and size variation in frugivores, in particular in long-lived taxa such as giant tortoises, when studying ingestion effects on the germination performance of plants.

Keywords

Ecological analogues, exotic species invasion, gut passage, plant–frugivore interactions, seed germination

Introduction

Rewilding with taxon substitutes, the intentional introduction of exotic species to resurrect lost ecosystem functions of recently extinct species, is currently hotly debated (Donlan *et al.* 2006; Rubenstein *et al.* 2006; Caro *et al.* 2009; Griffiths and Harris 2010; Hansen *et al.* 2010). Most proposals have been to replace extinct large herbivores with carefully selected exotic vertebrates, to resurrect grazing and seed dispersal functions (Zimov 2005; Donlan *et al.* 2005; Griffiths *et al.* 2010; Hansen *et al.* 2010). Frugivores can play key roles in the reproduction of plants by dispersing seeds to sites favourable for establishment, and by ingesting seeds, thereby altering germination and seedling establishment performance (Traveset 1998; Schupp *et al.* 2010). Fleshy-fruited plants, especially on islands, are particularly vulnerable to extinctions or strong declines of mutualistic interactions with frugivorous vertebrates

75 (Meehan *et al.* 2002; Kaiser-Bunbury *et al.* 2010; Griffiths *et al.* 2011; Wotton and
76 Kelly 2011). Recently, it has been shown that frugivore taxon substitutes can restore
77 some of these lost interactions and facilitate the recruitment of endangered fleshy-
78 fruited plants (Griffiths *et al.* 2011). However, there is a risk that introducing
79 frugivores could also facilitate recruitment and spread of exotic plants pervading most
80 ecosystems (Nentwig 2007). Given that invasions of exotic plant species pose major
81 threats to biodiversity and ecosystem functioning (Millennium Ecosystem Assessment
82 2005), particularly on oceanic islands (Denslow 2003; Caujapé-Castells *et al.* 2010),
83 it is important to identify and mediate the risks associated with the introduction of
84 taxon substitutes. Using germination trials to test the probability of exotic plants
85 germinating following ingestion by frugivorous substitutes allows conservation
86 managers to limit unwanted plant-substitute interactions. Such trials are analogous to
87 pre-release tests investigating potential non-target effects of a biological control agent
88 (Howarth 1991).
89 As the Mascarene Islands are at the forefront of rewilding (Marris 2009), we
90 chose the island of Rodrigues to investigate whether Aldabran giant tortoises,
91 *Aldabrachelys gigantea*, chosen to replace the extinct giant tortoises, *Cylindraspis*
92 species (Hansen *et al.* 2010), could facilitate the germination of exotic plants. We
93 assessed what effect ingestion (gut passage and deposition in faeces) by *A. gigantea*
94 had on the percentage of seeds germinating of four exotic fleshy-fruited plant species
95 in Rodrigues. Understanding what effect these taxon substitutes will have on the
96 germination and seedling establishment stage is crucial, as these stages ultimately
97 affect the invasion potential of an exotic plant (Vila and D'Antonio 1998; Pysek and
98 Richarson 2007).

The effect of ingestion on germination patterns may vary considerably among plant and animal species (Lieberman and Lieberman 1986; Jordano 1992; Nogales *et al.* 2005; Traveset *et al.* 2008; reviewed in Traveset 1998), and with age and size variation (Hatt *et al.* 2002). Indeed, such variation is likely to influence germination patterns as giant tortoises vary considerably in age and size among sub-adult and adult individuals (Grubb 1971). However, very little is known about the role of such variation in frugivores on seed germination. We investigated how variation between four exotic fleshy-fruited plant species, and among adult and sub-adult *A. gigantea* affected the germination percentage of ingested seeds.

Materials and Methods

Study site and study species

The experiment was conducted at François Leguat Reserve (63°22′11.24″E, 19°45′15.76″S) near Anse Quitor, in the southwest of Rodrigues between 19 May and 8 September 2009. The island experiences trade winds from subtropical anticyclones in winter (May to October) and a tropical climate in the summer (November to April). It has a mean annual rainfall of 1105 mm and temperatures range from a mean of 21° C in winter to 29° C in summer (Mauritius Meteorological Services).

We selected four widespread exotic fleshy-fruited plant species on the island (Table 1). *Wikstroemia indica* L. (Thymelaeaceae) is a tall herb that was introduced to Rodrigues in 1828. Its natural range extends from south-east Asia to Australia. *Lantana camara* L. (Verbenaceae) is a highly invasive shrub, listed among the 100 worst invasive organisms worldwide (Global Invasive Species Database 2011). It often grows in dense patches up to 1 m in height. It was introduced to Rodrigues in the 1920s (Cheke and Hume 2008) from tropical America, and today dominates the

124 vegetation of southern Rodrigues. *Veitchia merrillii* (Becc.) H.E. Moore (Arecaceae),
125 which originates from the Philippines, inhabits coastal and lowland forests of
126 Rodrigues. The tree species *Mimusops coriacea* (DC.) Miq. (Sapotaceae) originates
127 from Madagascar and was introduced to Rodrigues in 1800 (Rouillard and Guého
128 1999).

129 *Aldabrachelys gigantea* from the Aldabra Atoll of the Seychelles are the only
130 extant Indian Ocean giant tortoises. Males can reach a straight carapace length of
131 more than a meter and a weight of 300 kg, whereas females are generally
132 considerably smaller (Grubb 1971). Both sexes feed on a wide variety of plants and
133 fruits (Grubb 1971). Between 2006 and 2007, 490 *A. gigantea* were introduced to the
134 François Leguat Reserve in Rodrigues to resurrect lost ecosystem functions that were
135 likely performed by the extinct Rodriguan giant tortoises, *Cylindraspis vosmaeri* and
136 *C. peltastes* (Weaver *et al.* 2008). We used five adult tortoises (60–120 years old)
137 weighing between 75–180 kg and with a straight carapace lengths (SCL) of over 60
138 cm, and 22 sub-adults (10–15 years old) weighing between 20–30 kg with a SCL
139 between 35–50 cm. Sub-adult and adult tortoises were housed in separate enclosures
140 with similar plant compositions dominated by the grass *Cynodon dactylon* (L.) Pers.
141 None of the plant species studied grew in the enclosures and it was unknown whether
142 tortoises would consume them when introduced into natural ecosystems. We used
143 tortoise age class, which like body mass or size, is positively correlated with digestive
144 tract length and thus can be expected to be positively related with seed retention time
145 (e.g. Hatt *et al.* 2002; but see Franz *et al.* 2011; Sadeghayobi *et al.* 2011).
146
147 *Studying tortoise ingestion*

148 It is important to note that the germination experiment was designed to test our two
149 main research questions: (1) what are the consequences of ingestion by *A. gigantea* on
150 the seed germination of exotic fleshy-fruited species? (2) How does age-class and
151 variation among tortoise individuals affect seed germination? This was achieved by
152 feeding as many individual tortoises as possible with the available fruits. We
153 investigated the effect of ingestion and not the mechanisms involved in the ingestion
154 process (e.g. pulp-removal by gut passage, seed scarification, or the presence of faecal
155 material; Traveset *et al.* 2001, 2008), as assessing the impact of introducing a taxon
156 substitute on the germination of exotic species is more critical to current management
157 issues. However, more detailed analyses of which factors are involved in the ingestion
158 process could be a fruitful area of future research.
159 The effect of ingestion by *A. gigantea* was investigated by comparing the
160 germination percentage (i.e. the proportion seeds of the total number of sown seeds
161 that germinated during the experiment) of the four plant species when ingested and
162 when not ingested. Fruits ingested by a vertebrate frugivore will always be deposited
163 with faecal material, and studies investigating ingestion effects on seed germination
164 that do not consider faecal material miss a potentially important factor affecting the
165 seed germination and establishment (Traveset *et al.* 2001). Seeds that passed through
166 the tortoise gut and were deposited in faeces were defined as ingested (Traveset *et al.*
167 2001), while seeds that were sown in soil as whole fruits were defined as non-
168 ingested.
169
170 *Feeding of tortoises*
171 Fruits of each species were collected from plants at different locations and randomly
172 divided among the treatments: ingested by sub-adult and adult tortoises v non-

ingested. Fruits were fed to 22 sub-adult and five adult tortoise individuals between 24 April and 11 May 2009. Sub-adult tortoises were fed all four species, whereas adults received only *L. camara* and *M. coriacea* due to limited numbers of available fruits of the other two species. Roughly the same amounts of fruits of each species were offered to each tortoise. To assess how variation among individual tortoises affects germination, they were simultaneously fed a unique combination of 20-60 coloured, non-toxic plastic pellets (1–5 mm in diameter; Albert GmbH & Co. KG, 32257 Bünde, Nordrhein-Westfalen). This enabled the source of the faecal matter to be identified. Pellets were mixed with banana or papaya to encourage consumption. Faeces were collected twice a day, from which the seeds of the four species were removed and stored in the dark for 10–24 days before sowing , thereby allowing seeds to dry naturally. This allowed non-ingested and ingested treatments to be sown simultaneously, removing potential bias due to possible variable weather conditions that could influence germination. Storing of seeds for this short time period should not have significantly affected germination percentage, although we cannot totally rule out this possibility. The mean seed retention time, the average number of days between seeds being ingested and appearing in the faeces, was calculated.

Germination experiment

Ingested seeds and non-ingested whole fruits were sown according to species in 8cm-diameter pots filled with 400 ml of soil, between 19 May and 4 June 2009. Ingested seeds were sown in a layer of 100 ml of faeces from which they originated (i.e. faeces with the same tortoise identity as the seeds) and free of seeds of the focal plant species, whereas non-ingested whole fruits were sown in a layer of 100 ml of soil. Within each pot, eight ingested seeds or non-ingested whole fruits, respectively, for *V.*

198 *merrillii* and *W. indica* were sown. Six ingested seeds or whole fruits, respectively,
199 were sown for *L. camara* within each pot. For the multi-seeded *M. coriacea*, in which
200 the number of seeds per fruit varied, we sowed two whole fruits for the non-ingested
201 treatment, based on the mean number of seeds per fruit (4 seeds per fruit; Table 1),
202 and 8 seeds for the ingested treatments. Thus, the germination percentage of non-
203 ingested seeds of *M. coriacea* could only be estimated. However, the small standard
204 error (Table 1) indicates that this estimation was robust and any potential bias was
205 small.

206 Where the seeds could not be assigned to an individual tortoise, or insufficient
207 seeds were collected from an individual, we used seeds originating from a mixture of
208 faecal material within the same age class (sub-adult or adult). In total, we had three
209 replicates for each treatment (ingested vs. non-ingested) x plant species x tortoise
210 individual combination, and nine pot replicates for each treatment x plant species x
211 adult or sub-adult mixture combination. The pots were randomised and arranged in
212 three blocks (to account for potential spatial and associated microclimatic variability),
213 with each block containing an equal number of pots of all treatment types.

214 The pots were watered to keep the soil moist and checked daily for seed
215 germination, which we defined as the emergence of any part of the seedling. A
216 toothpick was placed beside each germinated seedling to avoid double-counting. Pots
217 were regularly weeded to remove non-focal species. Slug poison was used to prevent
218 herbivory by slugs. We did not assess seedling survivorship as only 10 out of 1169
219 seedlings died during the experiment. After four months the experiment was
220 terminated.

221 This research was conducted in accordance with current national legislation
222 and international guidelines on animal husbandry and welfare, and with permission of

223 the authority of the "Francois Leguat Tortoise and Cave Reserve". No animal was
224 force-fed or harmed in any way.
225
226 *Statistical analysis*
227 The germination percentage at the end of the experiment was analysed using a
228 generalized linear model with quasi-binomial errors and F-tests instead of chi-squared
229 to account for overdispersion (Crawley 2007). The fitted and error terms of the model
230 are shown in Table 2. Since the interaction term ingestion × plant species was
231 significant (Table 2), indicating variation of the ingestion effects among plant species,
232 we also fitted separate models for each plant species, as described above, but without
233 the explanatory variable plant species and its interactions. All analyses were carried
234 out with R 2.7.2 (R Development Core Team 2008). Arithmetic means ± 1 standard
235 error are reported.
236
237 ## Results
238 Seed retention time was shorter for sub-adult tortoises (13 ± 1 days) than for adults
239 (18 ± 2 days). Gut passage removed all the fruit pulp of the studied species, with the
240 exception of *L. camara*, for which fruit pulp was only partially removed. In contrast,
241 it took roughly 6–8 weeks for the fruit pulp to decompose from non-ingested seeds.
242 The overall percentage of germinated seeds (ingested and non-ingested seeds)
243 varied among species (Fig. 1; Table 2). On average, ingestion by tortoises (both adults
244 and sub-adults) enhanced the percentage germination (Fig. 1; Table 2). However, the
245 effect of ingestion differed among species (significant ingestion × species interaction,
246 Table 2). While ingestion increased the percentages of germinated seeds in *M.*
247 *coriacea* ($F_{1,13} = 19.77$, $P = < 0.001$) and *L. camara* ($F_{1,14} = 5.79$, $P = 0.030$),

germination percentages of *V. merrillii* ($F_{1,8} = 0.86$, $P = 0.382$) and *W. indica* ($F_{1,8} = 1.26$, $P = 0.262$) were not significantly altered by ingestion (Fig. 1). A higher percentage of seeds germinated when ingested by sub-adult than adult tortoises (Fig. 2; Table 2).

Discussion

Our aim was to predict if ingestion by *A. gigantea* could enhance the germination of four exotic fleshy-fruited plant species. To our knowledge, this is the first study investigating the consequences of frugivorous taxon substitutes on the performance of exotic, fleshy-fruited plants.

Effect of ingestion on seed germination of exotic plants

Ingestion by *A. gigantea* enhanced percentage germination of *M. coriacea* and *L. camara*, although the probability of *L. camara* germinating remained low. The fruit pulp, which can act as a barrier to germination often contains germination inhibitors, and can be a potential source of infection by pathogens and attract insect predation (Barnea *et al*. 1990; Izhaki and Safriel 1990; Liu *et al.* 2004). Thus, the observed fruit pulp removal following passage through giant tortoise's gut may have played an important role in the observed increased percentage of seed germination in these species. Further research using additional treatments, such as manual depulping, is needed to determine the exact mechanisms affecting seed germination following ingestion by *A. gigantea*.

The low germination percentage of *L. camara* and *W. indica* during the four months of the experiment is in agreement with findings from other studies (e.g. Vivian-Smith *et al.* 2006; Yiping *et al.* 2010). There is no evidence for strong seed

273 dormancy as a potential explanation (Vivian-Smith *et al.* 2006; Yiping *et al.* 2010).
274 Indeed, Vivian-Smith *et al.* 2006 found that, depending on the biotype of *L. camara*,
275 most or even almost all germination events over 12-months occurred within the first
276 four months.
277
278 *Effect of age variation*
279 Knowledge of the effect of age and size variability in a frugivore species on the
280 germination of seeds is limited (Traveset 1998). We found that giant tortoise age class
281 (positively correlated with size) can have pronounced effects on the germination
282 percentage of ingested seeds. Seed retention time of sub-adults was shorter than that
283 of adult tortoises. Thus, the strong effect of age class on seed germination may have
284 been mediated by different seed retention times for adult and sub-adult tortoises, with
285 longer retention times adversely affecting seed germination (Levey and Grajal 1991;
286 Murphy *et al.* 1993). Differences in adult and sub-adult diet can be eliminated as a
287 potential explanation (Murray *et al.* 1994), since they were largely identical. These
288 findings highlight the importance of considering individual variation among
289 frugivores of different age or size when investigating ingestion effects on seed
290 germination, e.g. by studying a sufficiently high number of individual frugivores and
291 including frugivore identity into the analyses. This is likely to be especially important
292 for long-lived species such as giant tortoises, which vary considerably in size between
293 age classes.
294
295 *Implications for conservation and restoration*
296 The germination and seedling establishment stages are particularly critical steps for an
297 exotic plant species to successfully colonize a new environment and ultimately

298 determine its invasion potential (e.g. Vila and D'Antonio 1998; Gosper *et al.* 2005;
299 Buckley *et al.* 2006; Pysek and Richardson 2007). Our finding that ingestion by *A.*
300 *gigantea* facilitated the germination of two fleshy-fruited exotic plant species,
301 including the highly invasive *Lantana camara*, emphasizes the importance of
302 conducting experiments to predict potential unintended effects of restoration
303 programmes using taxon substitutes. We recommend that conservation managers
304 minimise interactions between *A. gigantea* and *M. coriacea* fruits. Such precautions
305 may be unnecessary for *L. camara* given its low germination percentage following
306 ingestion, and for *V. merrillii* and *W. indica*, which were not more likely to germinate
307 following ingestion. While our study provides a first step to assessing the interaction
308 consequences between this exotic frugivore and exotic plants, further *in situ* research
309 is essential to investigate which fleshy-fruited plant species introduced tortoises will
310 consume, and to gain insights into how *A. gigantea* will alter the spatial seed dispersal
311 patterns of exotic plants. Moreover, whilst our findings show that this taxon substitute
312 can facilitate the germination of exotic plant species, it has recently been shown to
313 facilitate the recruitment of a critically endangered, endemic ebony species (Griffiths
314 *et al.* 2011).

315 It should also be considered that had the native giant tortoises, *Cylindraspis*
316 species, survived, they too may have had similar effects on the germination
317 probability of exotic fleshy-fruited plant species, as *Cylindraspis* tortoises were
318 similar in physiology and foraging behaviour to *A. gigantea*. Because exotic plants
319 pervade most ecosystems (Nentwig 2007), native frugivores commonly facilitate the
320 germination and spread of exotic plants (e.g., Gosper *et al.* 2005; Buckley *et al.*
321 2006). Indeed, Galápagos giant tortoises (*Chelonoidis nigra*), which are being re-
322 introduced to islands within the Galápagos archipelago, consume and disperse large

quantities of the introduced, highly invasive guava, *Psidium guajava* (Myrtaceae) (Blake *et al.* in press).

Our research highlights how low-cost experiments can be used to forecast potential negative impacts enabling adaptive management of the habitat and taxon substitute population to mitigate and minimise any possible negative outcomes. Further research into plant-tortoise interactions *in situ* is needed to corroborate the findings from this nursery-based experiment. We recommend that the use of giant tortoises and other taxon substitutes must be regularly assessed using a cost-benefit analysis, whereby the potential negative effects are weighed against the benefits and the risks to the indigenous biota of not restoring ecosystem functions to degraded habitats.

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466 **Tables**

467 **Table 1.** Fruit and seed characteristics of the exotic plant species. Mean (± 1 standard
468 error) of fruit and seed size (the longest diameter), and the number of seeds per fruit
469 (n=50 for *M. coriacea*, n=30 for the other three species).

Scientific name	Family	Fruit size [mm]	No. of seeds per fruit	Seed size [mm]
Wikstroemia indica	Thymelaeceae	8.1 ± 0.1	1 ± 0.0	5.8 ± 0.1
Lantana camara	Verbenaceae	4.4 ± 0.9	1 ± 0.0	4.3 ± 0.7
Veitchia merrillii	Arecaceae	31.4 ± 0.3	1 ± 0.0	18.8 ± 0.2
Mimusops coriacea	Sapotaceae	38.4 ± 0.8	4 ± 0.2	19.6 ± 0.2

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Table 2. Results of the generalized linear model testing for variation in the proportion of germinated seeds four months after sowing. Each explanatory variable was tested against its appropriate error term ("error"). "Composition" (factor with levels "mixture" vs. "tortoise id" was included in the models, since not all ingested seeds recovered from faeces could be assigned to an individual tortoise, and also some seeds originating from a mixture of faecal material within the same age class ("mix") where used (see methods section for detailed description of the experimental design). Significant results (*P*-value ≤ 0.05) are highlighted in bold.

Source of variation	Df	Error	Deviance	*F*-value	*P*-value
Block	2	R	2.82	0.20	0.823
Species (S)	3	S×T	2152.52	240.33	**<0.001**
Ingestion (ingested vs. non-ingested)	1	T	187.41	34.12	**<0.001**
Composition (mix vs. id)	1	T	0.9	0.16	0.690
Tortoise age	1	T	48.39	8.81	**0.007**
Tortoise id (T)	21	R	115.34	0.76	0.769
Species × ingestion	3	S×T	65.09	7.27	**0.001**
Species × composition	3	S×T	3.41	0.38	0.768
Species × age	1	S×T	3.26	1.09	0.307
Species × tortoise id	22	R	65.68	0.41	0.992
Residual (R)	449		3244.1		

505 **Figure legends**

506 **Figure 1.** Mean (± 1 standard error) cumulated percentage of germination for the
507 ingestion and the non-ingested treatment (control) four months after sowing of seeds.
508 In the ingestion treatment seeds were recovered from faeces and planted together with
509 faecal material, while for the non-ingested treatment seeds within fresh fruits were
510 planted.

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512 **Figure 2.** Mean (± 1 standard error) cumulated percentage of germination of seeds
513 ingested by adults and sub-adults four months after sowing.

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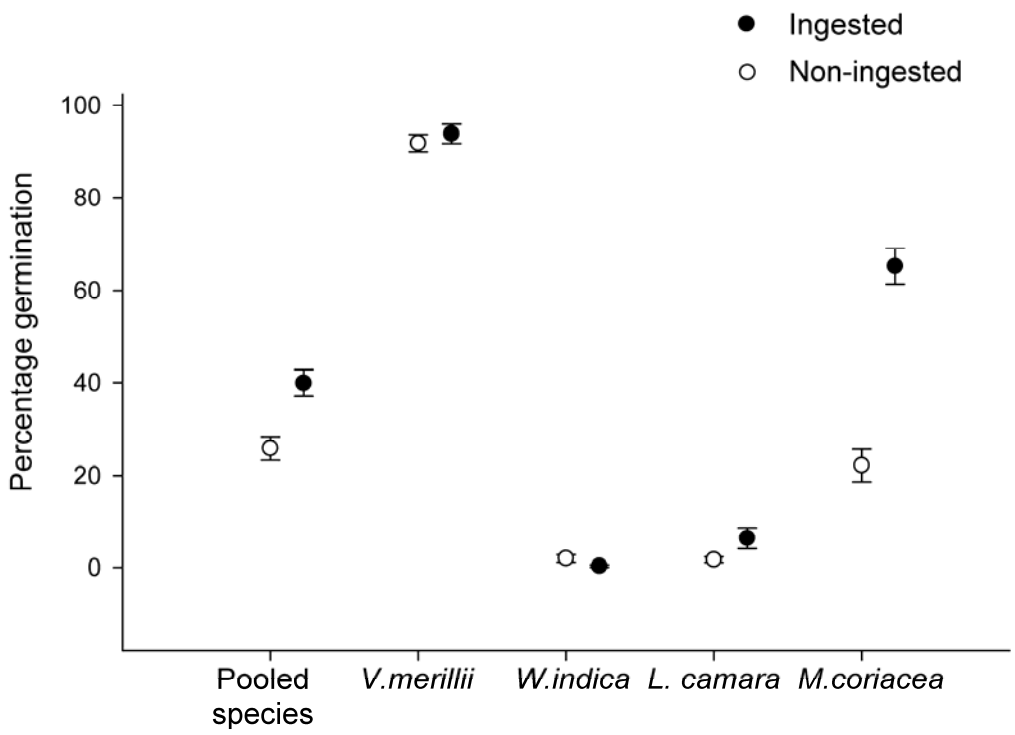
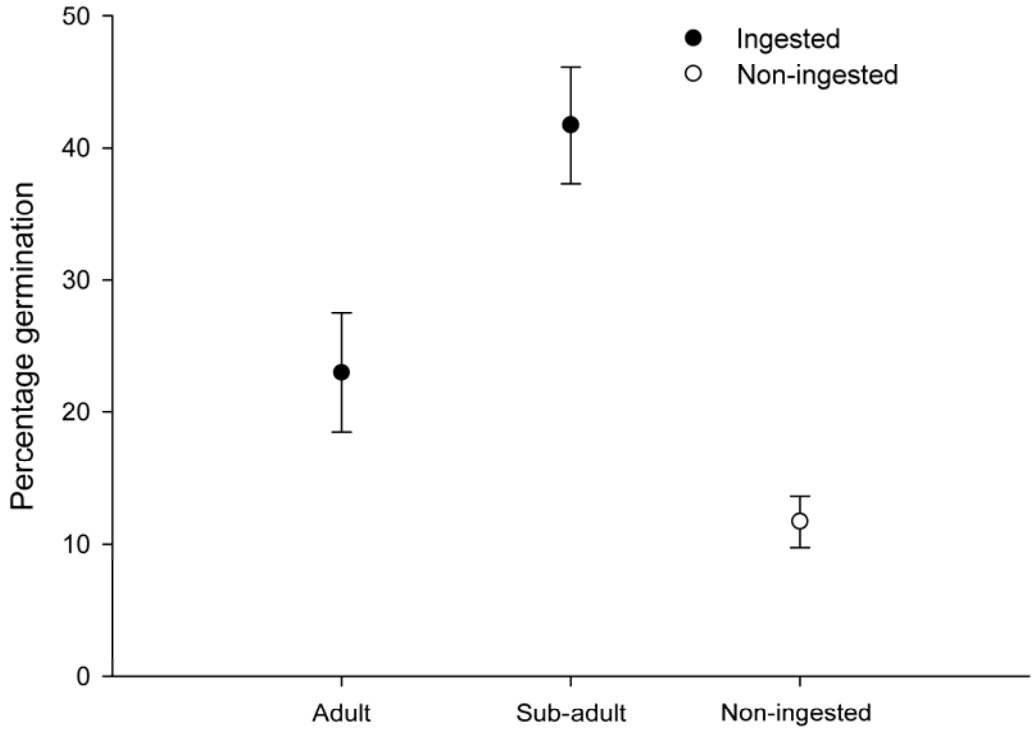
Figure 1


Ingested
Non-ingested
Percentage germination
100
80
60
40
20
0
Pooled species
V.merillii
W.indica
L. camara
M.coriacea

544 **Figure 2**



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